

ABSTRACT OF THE DISCLOSURE

A self-cleaving element for use in bioseparations has been derived from a naturally occurring, 43 kDa protein splicing element (intein) through a combination of protein engineering and random mutagenesis. A mini-intein (18 kDa) previously engineered for reduced size had compromised activity and was therefore subjected to random mutagenesis and genetic selection. In one selection a mini-intein was isolated with restored splicing activity, while in another, a mutant was isolated with enhanced, pH-sensitive C-terminal cleavage activity. The enhanced cleavage mutant has utility in affinity fusion-based protein purification. The enhanced splicing mutant has utility in purification of proteins such as toxic proteins, for example, by inactivation with the intein in a specific region and controllable splicing. These mutants also provide new insights into the structural and functional roles of some conserved residues in protein splicing. Thus, disclosed and claimed are: a genetic system and self-cleaving inteins therefrom; bioseparations employing same; protein purification by inactivation with inteins in specific regions and controllable intein splicing; methods for determining critical, generalizable residues for varying intein activity; and products

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